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(54) MONOCLONAL ANTIBODY AGAINST CARP VITELLOGENIN

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a monoclonal antibody which is specific to calp vitellogenin, and is hence useful for the determination of toxicity of a chemical substance, the estimation of environmental pollution, and the determination of the sex of the calp.
SOLUTION: This monoclonal antibody is specific to calp vitellogenin which is produced by a hybridoma (FERM P-16943). It is preferable to determine a concentration of calp vitellogenin by reacting the monoclonal antibody with vitellogenin in a humor of a calp. In addition, it is preferable to estimate the toxicity of chemical substances such as those having endocrine-disturbing effects or the environmental pollution by reacting the above-mentioned monoclonal antibody with vitellogenin in the humor of a calp, followed by using, as an index, an increase in the concentration of the vitellogenin determined by the reaction.

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CLAIMS

[Claim(s)]
[Claim 1] The monoclonal antibody to BITEROJIENIN of a carp.
[Claim 2] The monoclonal antibody to BITEROJIENIN of a carp produced by the hybridoma whose trust number is FERM P-16943.
[Claim 3] The density measurement approach of BITEROJIENIN of a carp characterized by making a monoclonal antibody and BITEROJIENIN in the body fluid of a carp according to claim 1 or 2 react.
[Claim 4] How to make a monoclonal antibody and BITEROJIENIN in the body fluid of a carp according to claim 1 or 2 react, and to evaluate the toxicity of a chemical, or environmental pollution by making into an index the rise of the concentration of BITEROJIENIN measured by this reaction.
[Claim 5] The approach according to claim 4 a chemical is what has an endocrine disruption operation.
[Claim 6] The approach according to claim 4 of being what environmental pollution depends on the chemical which has an endocrine disruption operation.
[Claim 7] The sex judging approach of the carp characterized by making a monoclonal antibody and BITEROJIENIN in the body fluid of a carp according to claim 1 or 2 react.
[Claim 8] The toxicity containing a monoclonal antibody according to claim 1 or 2 of a chemical, or the kit for evaluation of environmental pollution.
[Claim 9] The kit for a sex judging containing a monoclonal antibody according to claim 1 or 2 of a carp.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the toxicity of the chemical which used this antibody for the monoclonal antibody to BITEROJIENIN of a carp, and the list or the evaluation approach of environmental pollution, and the sex judging approach of a carp.
[0002]
[Description of the Prior Art] The abnormalities in reproduction of the animal considered that current and a chemical are the causes are observed in every corner of the earth. For example, the report of the abnormalities in reproduction in the United States of America and the APOPUKA lake of Florida is known best. According to the investigation report, many individuals which shrank from the magnitude of 1 / 4 - 1/2 with the normal penis of a male alligator were discovered, it compared normally in one of these, and a female alligator, and formation of the multi-**** follicle of the ovary and a polykaryotic egg was remarkable. It became clear that the cause was what is depended on the matter called JIKOHORU which is the DDT related substance taken out from the chemical plant located by the lake, and it has turned out that JIKOHORU is what has the same operation as a female sex hormone.
[0003] Thus, the chemical which acts on the endocrine system of a living thing and carries out the failure of the function is called endocrine disruptors (endcrine disruptors) (the so-called environmental hormone), and is known as matter in which an organochlorine compound, bisphenol A, an organometallic compound, etc. have an endocrine disruption operation until now. And it is suspected whether the matter with the operation same also in various strange compounds other than these matter exists.
[0004] The chemical with structure similar to the estrogen compound which is an original female sex hormone is that association to the receptor in the living body is shown, and causing a certain kind of hormone action is known. The example also caused many of abnormalities in a reproductive organ in the child born to 1950- 1970 from the mother who took DES (synthetic hormone drug) in the U.S. However, the chemical mentioned above was not necessarily structure similar to estrogen in many cases, and it was very difficult to predict as endocrine disruptors.
[0005] Conventionally, as an approach of evaluating an endocrine disruption operation of a chemical, yeast is made to discover a estrogen acceptor in a test tube, a mouse etc. is medicated with a chemical in the approach of measuring the degree which the compound concerned combines with the acceptor, or in the living body, and the approach of analyzing the generative function etc. is adopted. However, in the test method in the former test tube, effect of the metabolite of a chemical in the living body cannot be evaluated. Moreover, in a test method in the living body [latter], that use a lot of animals for observation and analysis, and the analysis takes a great effort etc. also has many problems.
[0006] By the way, if fishes etc. are bred under existence of the chemical which has an endocrine disruption operation, it is known that BITEROJIENIN which is a yolk protein precursor peculiar to a female will be guided also into a male living body (Steroids 1980 Mar;35(3):315-328, Le Menn F et al.). Therefore, the attempt which is going to evaluate the endocrine disruption operation which a chemical has has been made by measuring the concentration of BITEROJIENIN. However, since BITEROJIENIN was the unstable matter, the big problem was to measure correctly depending on instrumental analyses, such as high performance chromatography. Moreover, there is no approach used general-purpose also about immunological measurement, and the evaluation approach of the endocrine disruption operation which made induction of BITEROJIENIN the index is not established.
[0007]

[Problem(s) to be Solved by the Invention] This invention aims at offering the toxicity of the chemical which used this antibody for the monoclonal antibody to BITEROJIENIN of a carp, and the list or the evaluation approach of environmental pollution, and the sex judging approach of a carp.
[0008]
[Means for Solving the Problem] By using as an antigen the lipovitellin which is protein which constitutes a part of BITEROJIENIN, as a result of inquiring wholeheartedly, in order to solve the above-mentioned technical problem, this invention person succeeds in producing the monoclonal antibody which does not react to other related protein originating in the ovary which exists in body fluid, but reacts only to BITEROJIENIN specifically, and came to complete this invention.
[0009] That is, this invention is a monoclonal antibody to BITEROJIENIN of a carp. What is produced as this monoclonal antibody by the hybridoma whose trust number is FERM P-16943 is mentioned.
[0010] Furthermore, this invention is the approach of evaluating the toxicity of a chemical or environmental pollution characterized by making said monoclonal antibody and BITEROJIENIN in the body fluid of a carp reacting. As a chemical, what causes an endocrine disruption operation, for example is mentioned, and what is depended on the endocrine disruption operation which the chemical concerned produces owing to as environmental pollution is mentioned.
[0011] Furthermore, this invention is the sex judging approach of the carp characterized by making said monoclonal antibody and BITEROJIENIN in the body fluid of a carp react. Furthermore, this invention is a kit for evaluating the toxicity of a chemical or environmental pollution containing said monoclonal antibody. Hereafter, this invention is explained to a detail.
[0012]
[Embodiment of the Invention] This inventions are simple and a thing which makes it possible to measure to high sensitivity about BITEROJIENIN by making this monoclonal antibody react with BITEROJIENIN in the body fluid of a carp about the monoclonal antibody which can recognize specifically BITEROJIENIN which is yolk protein peculiar to the female of a carp. Consequently, this invention estimates the toxicity (especially reinforcement of an endocrine disruption operation) of a chemical, the contamination situation of the endocrine disruptors in a certain area is grasped, and it becomes possible to distinguish the sex of a carp further.
[0013] By carrying out immunity of the animal by using as an antigen the lipovitellin which constitutes a part of BITEROJIENIN, with other constituents of BITEROJIENIN which separates and exists in body fluid, this invention person did not react but thought that the monoclonal antibody which recognizes BITEROJIENIN specifically was obtained. I thought that measurement of BITEROJIENIN concentration was attained correctly, without being influenced of other related protein which originates in the ovary which exists in body fluid by this. Moreover, this invention person thought that the monoclonal antibody which recognizes BITEROJIENIN of a carp specifically could be obtained by using BITEROJIENIN of two or more fishes for screening. So, in this invention, while producing the monoclonal antibody to BITEROJIENIN, using this monoclonal antibody, it is simple and exact and the measuring method of high sensitivity BITEROJIENIN is established compared with a high speed liquid chromatography etc. And the toxic (endocrine disruption operation) reinforcement which a chemical has using the monoclonal antibody of this invention is examined. This reinforcement can be evaluated by measuring whether BITEROJIENIN concentration in the living body rises intentionally with the chemical which has an endocrine disruption operation.
[0014] Furthermore, this invention person thought that sex distinction of a carp was possible by making BITEROJIENIN concentration into an index. So, in this invention, the sex distinguishing method of a carp is established by making the above-mentioned monoclonal antibody react with BITEROJIENIN, and measuring BITEROJIENIN concentration.
[0015] 1. In order to carry out immunity of the animal in producing the monoclonal antibody of BITEROJIENIN and preparation this invention of a lipovitellin, and in order to choose the hybridoma which recognizes the lipovitellin of a carp specifically, it is necessary to prepare BITEROJIENIN and a lipovitellin. These protein is the followings, and can be made and prepared.
[0016] (1) Preparation BITEROJIENIN of BITEROJIENIN is the precursor of the phosphoprotein contained in the yolk, is made from liver in large quantities, and is secreted in blood. In this invention, a blood serum can be prepared being able to cover the blood extracted from the carp over centrifugal separation, and BITEROJIENIN can be refined by combining centrifugal separation, a gel-filtration

column chromatography, etc. suitably further. Refined BITEROJIENIN is used for screening of the hybridoma which produces the monoclonal antibody which recognizes BITEROJIENIN of a carp specifically etc.

[0017] (2) The preparation lipovitellin of a lipovitellin is a lipoprotein contained in the yolk. Therefore, a lipovitellin can be refined by combining centrifugal separation, a gel-filtration column chromatography, etc. from the yolk of a carp. The refined lipovitellin is used for the immunity for producing the antibody which recognizes BITEROJIENIN specifically.

[0018] 2. Although the monoclonal antibody of production this invention of the monoclonal antibody to BITEROJIENIN of a carp usually means the whole antibody molecule which can be combined with BITEROJIENIN of a carp, as long as it can combine with BITEROJIENIN of a carp, it may be the fragment (for example, Fab or F(ab')2 fragment). The monoclonal antibody of this invention can be manufactured with either of the various approaches. The manufacturing method of such an antibody is Jet al., [, for example, Sambrook, which is common knowledge in the field concerned, Molecular Cloning, and Cold Spring Harbor Laboratory Press (1989) Reference]

[0019] (1) Medicate mammalian, for example, a rat, a mouse, a rabbit, etc. by using as an antigen the lipovitellin of the carp which is the extraction above of an antibody forming cell, and was made and prepared. The dose per animal of an antigen is 0.1-100mg, when not using an adjuvant, and when using an adjuvant, it is 1-100microg. As an adjuvant, the Freund's complete adjuvant (FCA), the Freund's incomplete adjuvant (FIA), hydroxylation aluminium adjuvant, etc. are mentioned. Immunity is mainly performed by pouring into hypodermically, intraperitoneal, etc. in a vein. moreover, especially spacing of immunity is limited -- not having -- several week spacing from several -- it is two - five-week spacing preferably, and immunity is performed 2 to 5 times preferably 1 to 10 times. And an antibody forming cell is preferably collected one - 14 days after one - 60 days after the last immunity day. As an antibody forming cell, although a spleen cell, a lymph gland cell, a peripheral blood cell, etc. are mentioned, a spleen cell or a partial lymph gland cell is desirable.

[0020] (2) In order to obtain a cell fusion hybridoma, perform the cell fusion of an antibody forming cell and a myeloma cell. Generally [animals, such as a mouse,] as a myeloma cell united with an antibody forming cell, an available established cell line can be used. As for the cell strain to be used, what has the drugs selectivity which can survive only in the condition of could not survive in the HAT selective medium (hypoxanthine, aminopterin, and thymidine being included) in the state of un-uniting, but having united with the antibody forming cell is desirable. as a myeloma cell -- for example -- Mouse myeloma cell strains, such as P3X63-Ag.8.U1 (P3U1) and NS-I, are mentioned.

[0021] Next, the cell fusion of the above-mentioned myeloma cell and the antibody forming cell is carried out. Cell fusion mixes 1x106 to 2.5x106 antibody forming cells/ml, and 2x105 to 2x106 myeloma [/ml] cells in culture media for animal cell culture, such as DMEM which does not contain a blood serum, and RPMI-1640 culture medium, (the cell ratio 5:1 of an antibody forming cell and a myeloma cell is desirable), and performs a fusion reaction under cell fusion accelerator existence. As a cell fusion accelerator, a polyethylene glycol with a mean molecular weight of 1000-6000dalton etc. can be used. Moreover, an antibody forming cell and a myeloma cell can also be united using the cell fusion equipment of marketing using electrical stimulation (for example, electroporation).

[0022] (3) Sort out the hybridoma made into the purpose from the cell after sorting of a hybridoma, and cloning cell fusion processing. as the approach -- cell suspension -- for example, fetal-calf-serum content RPMI-1640 culture medium etc. -- suitable -- after dilution and a microtiter plate top -- 3x105 pieces / well extent firewood -- each -- a selective medium is added to a well and it cultivates by exchanging selective media suitably henceforth. Consequently, the cell grown after culture initiation and from around the 14th by the selective medium can be obtained as a hybridoma.

[0023] Next, it screens whether the target antibody exists in the culture supernatant of the increased hybridoma. Screening of a hybridoma is not limited especially that what is necessary is just to follow the usual approach. For example, a part of culture supernatant contained in the well grown as a hybridoma can be collected, and it can screen with enzyme immunoassay, radioimmunoassay, etc.

[0024] Cloning of syncytium is performed by limiting dilution etc. and establishes the hybridoma which is finally a monoclonal antibody production cell. 1G2, two A2, two A6, and 2H8 are obtained as a hybridoma which produces the monoclonal antibody of this invention as mentioned above. Among these, 1G2 call "Mouse-Mouse hybridoma C-1G2", and they are deposited with National Institute of Bioscience and

Human-Technology, Agency of Industrial Science and Technology (1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken) as FERM P-16943 on August 18, Heisei 10.
[0025] (4) As an approach of extracting a monoclonal antibody, a usual cell culture method or the usual ascites forming method etc. is employable from the hybridoma in which the monoclonal antibody carried out extraction establishment. In a cell culture method, a hybridoma is cultivated for seven - 14 days by the usual culture condition (for example, 37 degrees C, 5% CO2 concentration) in animal cell culture culture media, such as 10% fetal-calf-serum content RPMI-1640 culture medium, an MEM culture medium, or a serum free medium, and an antibody is acquired from the culture supernatant.
[0026] In the case of the ascites forming method, intraperitoneal [of the mammalian of the myeloma cell origin and an of-the-same-kind system animal] is medicated with about 1x107 hybridomas, and it proliferates a hybridoma in large quantities. And ascites is collected after one - two weeks. When purification of an antibody is needed in the extraction approach of the above-mentioned antibody, it can refine by choosing suitably well-known approaches, such as an ammonium-sulfate salting-out method, an ion exchange chromatography, gel filtration, and affinity chromatography, or combining these.
[0027] Although the monoclonal antibody of this invention reacts specifically with BITEROJIENIN of a carp, BITEROJIENIN of other kinds, such as a red sea bream and MAMICHOGU, has the property in which it does not react. Moreover, the subtype of the monoclonal antibody of this invention is IgG1 as a result of a commercial typing kit's investigating.
[0028] 3. In density measurement approach this invention of BITEROJIENIN, concentration of BITEROJIENIN of the body fluid (for example, plasma, a blood serum, etc.) of a carp can be measured using said monoclonal antibody (quantum). For example, after coating a plate with the blood serum of a carp, said monoclonal antibody is added and is made to react. The anti-mouse IgG antibody which carried out the horseradish peroxidase (HRP) indicator further is combined with the monoclonal antibody of this invention on a plate after washing a plate. Then, the BITEROJIENIN concentration in a sample can be measured by making a chromophoric substrate add and color and measuring an absorbance.
[0029] 4. In the toxicity of a chemical, or evaluation this invention of environmental pollution, the toxicity of a chemical or environmental pollution can be evaluated by making into an index the rise of the concentration of said BITEROJIENIN measured by carrying out like 3. For example, after breeding a male carp under existence of the chemical used as the candidate for evaluation, extract blood etc., BITEROJIENIN contained in a sample is made to react with the monoclonal antibody of this invention, and BITEROJIENIN concentration is measured. The size of the obtained measured value can estimate the toxicity (for example, reinforcement of an endocrine disruption operation) of a chemical. That is, a male carp is bred under existence of the chemical (for example, bisphenol A, phthalic ester) used as the candidate for evaluation, the BITEROJIENIN concentration in a blood serum is measured with time, and it compares with the BITEROJIENIN concentration in the blood serum obtained from the carp of contrast. Consequently, the chemical with which BITEROJIENIN concentration serves as a candidate for evaluation when high as compared with contrast is judged to have an endocrine disruption operation.
[0030] Moreover, it is possible by measuring the BITEROJIENIN concentration when breeding under the estrogen existence of a certain concentration, and the BITEROJIENIN concentration when breeding under the chemical existence used as the candidate for evaluation to relativize the reinforcement of an endocrine disruption operation of the chemical used as the candidate for evaluation with estrogen, and to evaluate it. That is, rather than BITEROJIENIN concentration when the BITEROJIENIN concentration when breeding under the estrogen existence of a certain concentration (for example, 100 ppm) breeds under existence of the chemical the reinforcement of an endocrine disruption operation is unknown and is [chemical] estrogen and this concentration (for example, 100 ppm), when it is 1/10, an endocrine disruption operation of the chemical concerned can be judged to be 10 times stronger to estrogen. In addition, BITEROJIENIN concentration can be measured by said approach of 3.
[0031] 5. In approach this invention which evaluates the environmental pollution condition by the chemical which has an endocrine disruption operation, the contamination condition of the environment by the chemical which has an endocrine disruption operation can be evaluated by making BITEROJIENIN in the body fluid of the carp of the male which inhabits the river used as the candidate for evaluation, a lake, etc. react with the monoclonal antibody of this invention, and measuring the concentration of BITEROJIENIN. The measurement result can judge that the environment is polluted with the chemical which has a certain endocrine disruption operation, when high as compared with contrast. In addition,

BITEROJIENIN concentration can be measured by said approach of 3.
[0032] 6. In approach this invention which distinguishes the sex of a carp, it is possible by making the aforementioned monoclonal antibody react with BITEROJIENIN in the body fluid of a carp, and measuring BITEROJIENIN concentration to distinguish the sex of a carp. For example, the blood of a carp is extracted, and if can measure the BITEROJIENIN concentration, it cannot be detected and a male and concentration are high, it can be judged as a female. In addition, BITEROJIENIN concentration can be measured by said approach of 3. However, in order to carry out the sex judging of a carp, it is required to use the carp captured from a laboratory or a nursery etc. which is not polluted with a chemical.
[0033] 7. The toxicity of a chemical, the object for evaluation of environmental pollution, or the monoclonal antibody of kit this invention for a sex judging of a carp can be diluted with the well-known buffer solution (pH 7-8) of 1M-10M, can be made into 0.01 microg/ml - 1microg [/ml] concentration, and can be used as the toxicity of a chemical, the object for evaluation of environmental pollution, or a kit for a sex judging of a carp. As the buffer solution, a phosphate buffer solution, the McLaren buffer solution, tris buffers, veronal buffer solution, the glycine buffer solution, etc. are mentioned.
[0034] Moreover, the monoclonal antibody of this invention can also be fixed and used for support. Sepharose etc. is mentioned as support. For example, it can be used by combining the monoclonal antibody of this invention with support, and making it distribute in the buffer solution, or stuffing a column.
[0035]
[Example] Hereafter, an example explains this invention still more concretely. However, as for this invention, the technical range is not limited to these examples.
[an example 1] -- preparation (1) of a monoclonal antibody the female of (Preparation i) BITEROJIENIN vitellogenic stage of an antigen -- 7ml of blood serums extracted from the carp -- 0.02M After dialyzing to the potassium phosphate buffer solution (pH 6.9) overnight, it added in the hydroxyapatite column (35ml) which equilibrated with this buffer solution. After obtaining the passing material drawing by the 0.02M potassium phosphate buffer solution, it was eluted in the stepwise technique with the potassium phosphate buffer solution of 0.05M, 0.1M, and 0.2M and 1.2M. 1.2M It added in the Sepharose 6B column after condensing the elution fraction by the potassium phosphate buffer solution. collecting symmetry peaks -- purification -- a carp -- BITERO Jenin was able to be obtained.
[0036] (ii) It is equivalent **** and 0.1M about tris buffers (pH 8.0) in 20g of eggs of the carp of a lipovitellin vitellogenic stage. It dialyzed to the potassium phosphate buffer solution. After carrying out at-long-intervals alignment separation of this by 10,000 rpm for 20 minutes, supernatant liquid was collected and it added in the hydroxyapatite column which equilibrated with this buffer solution. 0.1M and 0.4M, And with the potassium phosphate buffer solution of 1.2M, it was eluted in the stepwise technique. 0.4 M It added in the Sepharose 6B column after condensing the elution fraction by the potassium phosphate buffer solution. The obtained lipovitellin is used for production of the monoclonal antibody of this invention as an antigen.
[0037] (2) After mixing the immunity 0.5 mg of animal/ml antigen (lipovitellin) with the equivalent Freund adjuvant and producing an emulsion, immunity was carried out the animal 200micro everyl. /into the back hide of a Balb/c mouse. The booster (200microl./(animal)) was performed every two weeks, after carrying out immunity 3 times from a priming, it collected blood from the eye socket, and antibody titer was checked.
[0038] (3) a carp with a measurement [of antibody titer] of 5microg [/ml] -- the PBS solution containing a lipovitellin -- every [100microl/well] -- in addition to the ELISA plate, at 4 degrees C, it put overnight and solid-phase-ized. BURROKKINGU [Tween-PBS containing 5mg //ml / BSA was added 200microl/well, and / the room temperature / it put for 1 hour and]. Then, the antiserum diluted with Tween-PBS containing 1mg [/ml] BSA was added 100microl/well, and was made to react at a room temperature for 2 hours. Furthermore, the HRP indicator anti-mouse IgG goat antibody diluted with Tween-PBS containing 1mg [/ml] BSA was added 100microg/well, and was made to react at a room temperature for 1 hour. Next, 2mg/ml OPD (o-phenylenediamine dihydrochloride) was added 100microl/well, the coloring reaction was performed for 3 minutes at the room temperature, and the absorbance of 492nm was measured with the microplate reader.
[0039] (4) The cell fusion of the spleen cell of the mouse with which the rise of the cloning antibody

titer of cell fusion and an antibody production hybridoma was checked, and myeloma cell P3U1 was carried out by the polyethylene-glycol method at a rate of 5:1, and selective culture of a hybridoma was performed by the HAT selective medium. the technique of having collected hybridoma culture supernatants on the 10th day of the cell fusion, and having measured antibody titer, and the same technique -- ELISA -- carrying out -- a carp -- the reactivity with BITERO Jenin screened the stock which is a positivity.

[0040] One after measuring the number of cells of a hybridoma which became a positivity by the above-mentioned screening It wound around 96 well plate and was crowded so that it might be set to an individual/well (subcloning), and only the well of a single colony screened again ten days after. Similarly subcloning was again performed about the hybridoma which became a positivity by screening, and screening actuation was continued until the property was in agreement 100%.

[0041] consequently, a carp -- four shares (1G2, two A2, two A6, 2H8) of specific antibody forming cells were obtained to BITERO Jenin. Moreover, the subtype of the monoclonal antibody of this invention checked that it was IgG1 with the commercial typing kit. In addition, hybridoma 1G2 (name : "Mouse-Mouse hybridoma C-1G2") are deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken) as FERM P-16943 on August 18, Heisei 10.

[0042] [an example 2] -- the examination about species-specific [of the monoclonal antibody of this invention] -- a carp -- monoclonal antibody production cell strain 1G2 to BITERO Jenin of this invention, two A2, two A6, and 2H8 were examined about species-specific. BITEROJIENIN of a carp used as an antigen was refined from the plasma of Metz. Moreover, a red sea bream, MAMICHOGU, and BITEROJIENIN of a cyprinodont were refined from the plasma of the male processed by 17beta-estradiol.

[0043] various kinds of BITEROJIENIN -- respectively -- 5microg [/ml] concentration -- 2mM PBS (pH 7.4) -- dissolving -- every [50microl/well] -- the ELISA plate was coated. Then, Tween-PBS which contains BSA 0.5% was 200micro-l/well-added, and was blocked. After carrying out phase dilution from ml in 2microg /, carrying out 50microl/well addition and making a purification antibody react for 2 hours, the HRP indicator anti-mouse IgG goat antibody diluted with Tween-PBS containing 1mg [/ml] BSA was added 100microg/well, and was made to react at a room temperature for 1 hour. Next, 2mg/ml OPD (o-phenylenediamine dihydrochloride) was added 100microl/well, the coloring reaction was performed for 3 minutes at the room temperature, and the absorbance of 492nm was measured with the microplate reader.

[0044] Consequently, it checked that, as for BITEROJIENIN of other kinds, the monoclonal antibody of this invention did not show reactivity although BITEROJIENIN of a carp reacts (drawing 1 -4). Therefore, it turned out that four sorts of monoclonal antibody production cells of this invention are hybridomas which produce a specific antibody to BITEROJIENIN of a carp.

[0045] [Example 3] In order to measure BITEROJIENIN in a sample using the monoclonal antibody of examination this invention of the system of measurement of BITEROJIENIN, the sandwiches ELISA method was applied. namely, the phosphate buffer solution (PBS) solution containing the 5microg [/ml] monoclonal antibody of this invention produced by hybridoma 1G2 -- every [100microl/well] -- it put in and coated with 4 degrees C overnight. After removing an antibody solution, it blocked for 1 hour by T-PBS which contains BSA 0.5% after 3 times washing by Tween-PBS (T-PBS) 0.05%. the purification which carried out phase dilution by Tween-PBS which contains 1mg/ml BSA after washing -- a carp -- a lipovitellin -- every [100microl/well] -- it put in and was made to react at a room temperature for 2 hours 2microg/mlHRP indicator after repeating the same washing actuation as the above 3 times -- anti- -- a carp -- a BITERO Jenin rabbit IgG antibody -- every [100microl/well] -- in addition, it reacted at the room temperature for 1 hour.

[0046] citrate buffer solution which contains 2 mg/ml OPD (o-phenylenediamine) after repeating the same washing actuation as the above 5 times (pH5.0) every [100microl/well] -- it put in and was made to react for 15 minutes After the reaction, 50microl/well addition of the 2-N sulfuric acid was carried out, and the stop and the absorbance of 490nm were measured for the reaction. Consequently, in this system of measurement, a measurement limitation is 9.8x10 to 4 microg/ml, and it was checked that it is possible to measure BITEROJIENIN of a carp to high sensitivity (drawing 5).

[0047] [Example 4] Reactant this example of the monoclonal antibody of this invention examined the

reactant difference between the monoclonal antibody of this invention obtained by carrying out immunity of the lipovitellin which is a part of structure of BITEROJIENIN, and the monoclonal antibody obtained by carrying out immunity of BITEROJIENIN of all structures. The reactivity of each antibody was investigated by the following approaches using the monoclonal antibody (1G2) of this invention, and the monoclonal antibody (3 A4) obtained by carrying out immunity of whole BITEROJIENIN by the same approach as an example 1 (Western blotting).

[0048] Electrophoresis was performed for the plasma obtained from various kinds of fish by acrylamide gel 7%, and the protein on gel was imprinted to the nitrocellulose membrane. The imprinted nitrocellulose membrane was shaken at the room temperature in the block ace (Snow Brand Milk Products) for 1 hour. Then, the nitrocellulose membrane was dipped in the antibody solutions (1G2 and 3 A4) of each diluted with the dilution block ace 10 times, and it shook at the room temperature for 2 hours. The nitrocellulose membrane was shaken for 10 minutes to the Tween content PBS (T-PBS) 0.05%, and the film was washed. Furthermore, T-PBS was exchanged and same washing actuation was performed twice. The nitrocellulose membrane was dipped in the HRP indicator anti-mouse IgG goat antibody solution diluted with the dilution block ace 2500 times 10 times after membranous washing, and it shook at the room temperature for 1 hour. Then, washing actuation of the film was performed like the above. After being the tris sodium chloride buffer solution and rinsing a nitrocellulose membrane, the antigen-antibody reaction was detected using the ECL detection system (Amersham) (drawing 6).

[0049] Consequently, it was that to which other components in plasma do not react to, but the monoclonal antibody of this invention reacts only to BITEROJIENIN (drawing 6 , an upper case, lane 2). On the other hand, the monoclonal antibody obtained by carrying out immunity of BITEROJIENIN of all structures reacted also to components in plasma other than BITEROJIENIN (drawing 6, the lower berth, lane 1), and it became clear that singularity became low. In each panel of drawing 6, the lanes 1-4 of an upper case express the sample of a red sea bream, a carp, MAMICHOGU, and the cyprinodont origin, respectively, and the lanes 1-4 of the lower berth express the sample of a carp, a red sea bream, MAMICHOGU, and the cyprinodont origin, respectively. From the above thing, it was shown that the monoclonal antibody of this invention is more useful as a reagent for density measurement of BITEROJIENIN since other components in plasma have high singularity [as opposed to / do not react and / BITEROJIENIN].

[0050] [an example 5] -- the carp which inhabits a river using the monoclonal antibody of this invention in measurement this example of the BITEROJIENIN concentration in a blood serum of the carp which inhabits a river -- the BITEROJIENIN concentration in a blood serum was investigated. The carp was captured in Japan 8 rivers and the blood of 101 individuals distinguished as it is a male was extracted. After obtaining a blood serum, BITEROJIENIN concentration was measured by the same approach as an example 3.

[0051] Consequently, although the BITEROJIENIN concentration in a blood serum was 0.1micro less thang/ml in many individuals, many individuals which show a high price depending on a river were seen (drawing 7, River D). eight rivers (river A-H) made applicable to investigation -- the BITEROJIENIN concentration in a blood serum was independently shown in drawing 7 as a distribution map for every individual.

[0052]

[Effect of the Invention] The toxic measuring method of the chemical which used this antibody for the monoclonal antibody to BITEROJIENIN of a carp and the list by this invention, the evaluation approach of environmental pollution, and the sex judging approach of a carp are offered. It is specific to a carp, and since the monoclonal antibody of this invention recognizes a lipovitellin part among the structures of BITEROJIENIN, it is not influenced of the related protein originating in other ovaries, but exact measurement is possible for it. Therefore, the monoclonal antibody of this invention is useful to the toxic measuring method of a chemical, the evaluation approach of environmental pollution, and the sex judging approach of a carp.

[Translation done.]

* NOTICES *

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3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing species-specific [of the monoclonal antibody which hybridoma 1G2 produce].
[Drawing 2] It is drawing showing species-specific [of the monoclonal antibody which a hybridoma two A2 produces].
[Drawing 3] It is drawing showing species-specific [of the monoclonal antibody which a hybridoma two A6 produces].
[Drawing 4] It is drawing showing species-specific [of the monoclonal antibody which hybridoma 2H8 produce].
[Drawing 5] It is drawing showing the reactivity over BITEROJIENIN of the carp in the sandwiches ELISA method of the monoclonal antibody which hybridoma 1G2 produce.
[Drawing 6] It is drawing showing the test result of Western blotting.
[Drawing 7] It is drawing showing the measurement result of the BITEROJIENIN concentration in a blood serum of the carp which inhabits a river.

[Translation done.]

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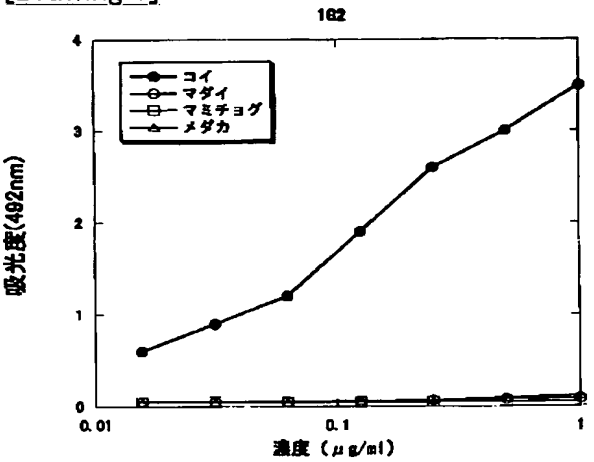
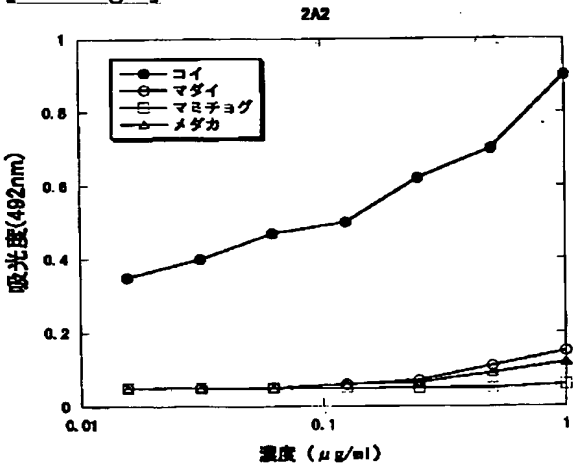
DRAWINGS

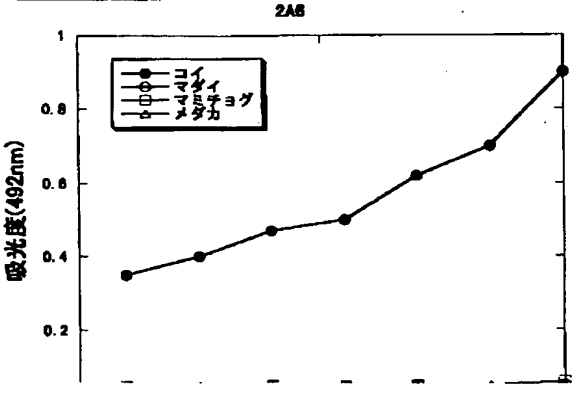
[Drawing 1]

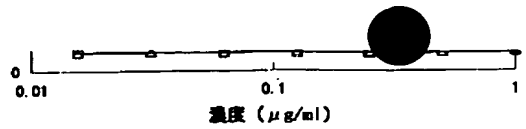


[Drawing 2]

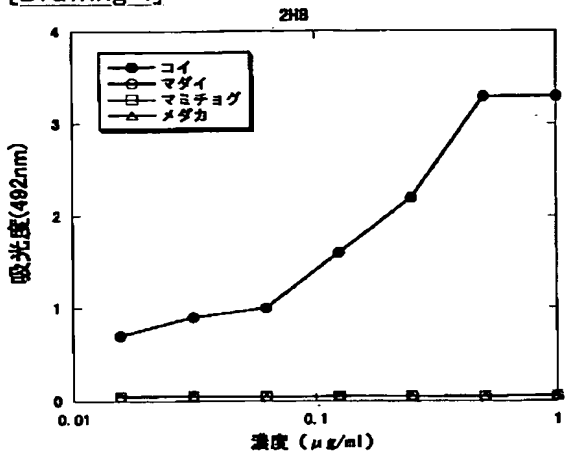


[Drawing 3]

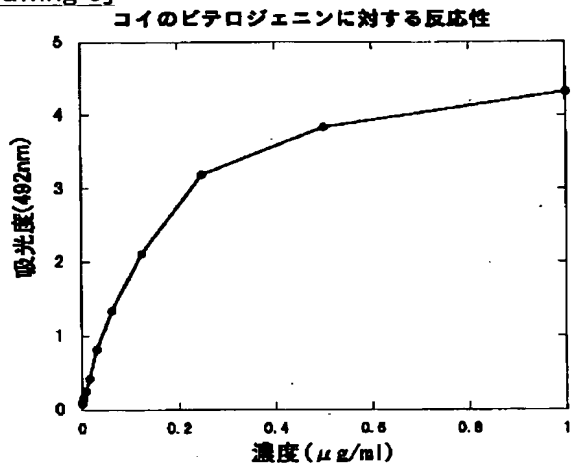




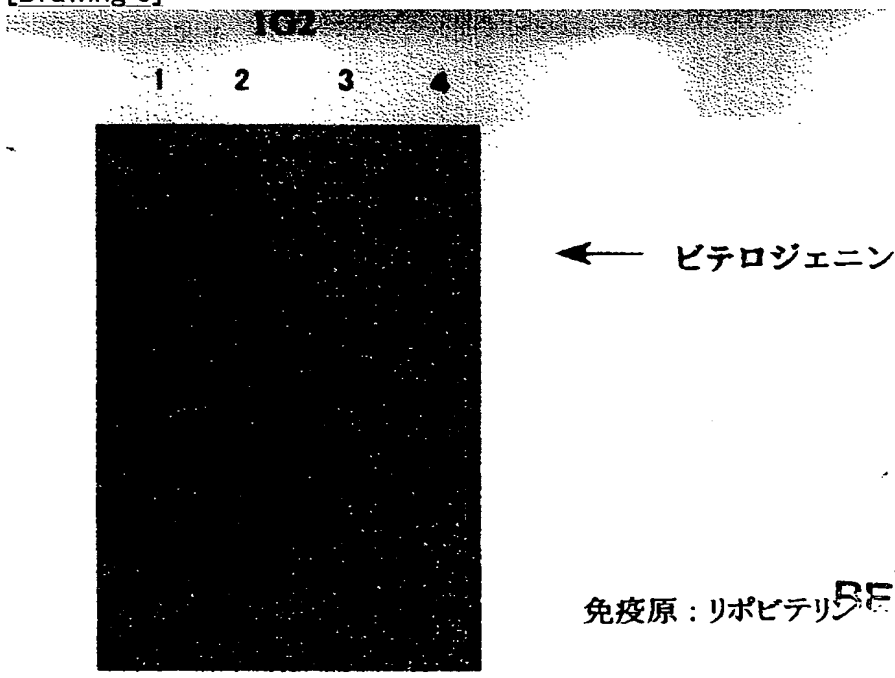
[Drawing 4]

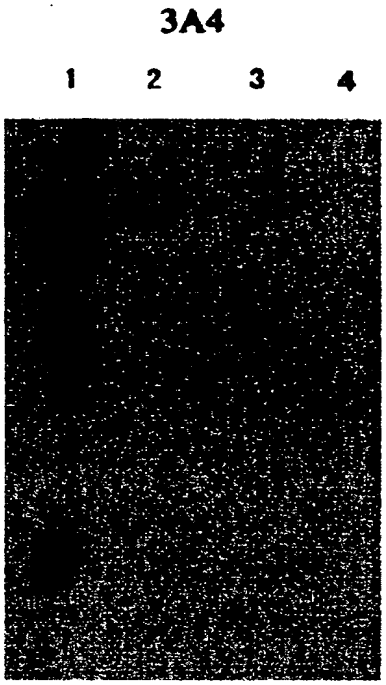


[Drawing 5]



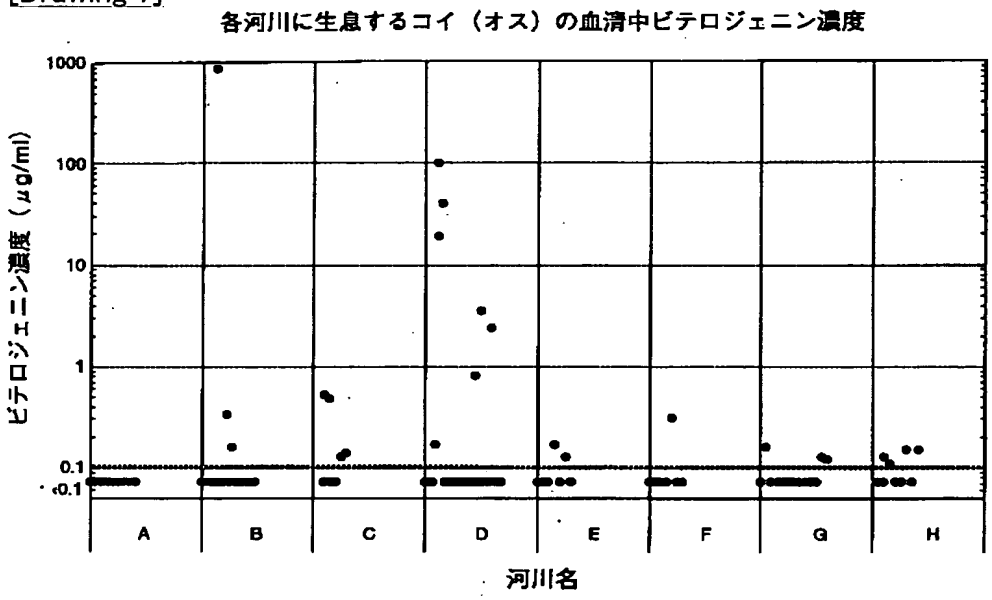
[Drawing 6]





免疫原：ビテロジェニン

[Drawing 7]



[Translation done.]